

# **SAFETY AND QUALITY EVALUATION OF A YOGURT-BASED DRINK PROCESSED BY A PILOT PLANT PEF SYSTEM**

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## **ABSTRACT**

*Yogurt-based drink samples were prepared by mixing plain yogurt, water, sugar, and strawberry flavored fruit syrup. The samples were treated by both the mild heat (60C for 30 s) combined with a pilot plant pulsed electric field (PEF) system and the mild heat only in order to determine changes in the product safety and quality. Changes in the product safety were measured as microbial count, and changes in the product quality as L, a, b, °Brix and pH values. The treated and control samples were stored at 4 and 22C for microbial, physical and sensory evaluations. Since the 60C-treated samples exploded after 14-day storage, the safety and quality evaluations for 91 days were performed for the*

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*60C+PEF and control samples, only. Microbial count of the 60C+PEF samples was significantly lower than that of the control samples at the two temperatures for the 91-day storage ( $P < 0.05$ ). There was no significant difference in the L, a, b values, °Brix and pH between the control and 60C+PEF samples ( $P < 0.05$ ). The control and 60C+PEF samples revealed no significant difference in the selected sensory attributes ( $P < 0.05$ ).*

## INTRODUCTION

Pulsed electric field (PEF) treatment is a promising nonthermal processing method to inactivate microorganisms (Jeyamkondan *et al.* 1999) and meet consumer demands for fresh-like quality foods. PEF-treatment of liquid foods such as apple juice, orange juice, cranberry juice cocktail, whole milk, 2% milk, skim milk, and chocolate milk is well established in the literature to provide a better quality product by the inactivation of spoilage and pathogenic bacteria than the traditional heat pasteurization (Dunn and Pearlman 1987; Qiu *et al.* 1988; Reina *et al.* 1988; Zhang *et al.* 1995; Barbosa-Canovas *et al.* 1999; Jin and Zhang 1999; Evrendilek *et al.* 1999, 2000, 2001; Yeom *et al.* 2000). Examples of the PEF-treated products delivered to the market include milk, fruit juices, beer, wine, cider, and a beverage containing PEF-treated orange juice (Jamieson and Williamson 1999; Swientek 1999). However, extending the use of this technology necessitates the application of PEF-treatment to particulate and high viscosity foods.

Yogurt beverages, generally from reduced solids, are pourable at the refrigerator temperatures and contain all natural vitamins and minerals of the milk and yogurt (Tamime and Deeth 1980). Yogurt, converted from milk, is much less prone to spoilage than nonfermented milk. The low pH due to the production of lactic acid during the fermentation inhibits the growth of most spoilage microorganisms but that of yeasts and molds. Yeasts and molds are, thus, the most likely types of the spoilage organisms that cause problems in yogurt as well as yogurt drinks (Suriyarachchi and Fleet 1981). The process of manufacturing and the addition of fruit and flavoring are the major operations that increase the risk of yeast contamination in yogurt drinks (Mckay 1992; Deak and Beuchat 1996). Hygienic practices, heat treatment, refrigerated storage, and antimicrobial agents are currently common applications used in the control of yeast spoilage of dairy products (Marth 1987).

Consumers are increasingly becoming health-conscious, thus demanding for fresh-like quality foods, extended shelf-life, and no artificial preservatives (Barsotti and Cheftel 1999; Lewis and Dale 1994; Russell *et al.* 1999). In order to determine the potential of PEF treatment for high viscosity food products and the extension of shelf-life of yogurt-based drinks without any significant adverse

impact on their physical and sensory properties, the interaction of mild heat and PEF treatments as a promising alternative to the thermal process needs to be explored.

The objective of this study was to assess the applicability and effectiveness of the mild heat (60C for 30 s) treatment combined with a pilot plant PEF treatment for the microbial, sensory and physical attributes of a yogurt-based drink stored at 4 and 22C.

## MATERIALS AND METHODS

### Preparation of Samples

Yogurt-based drink samples were prepared by mixing plain yogurt, water, sugar, and strawberry flavored fruit syrup in a stainless steel tank before being processed (Table 1). Flavored strawberry syrups were obtained from Stearns & Lehman (Mansfield, OH). The other ingredients were purchased from local grocery stores (Columbus, OH).

TABLE 1.  
FORMULATION OF THE YOGURT-BASED DRINK

Ingredients	Weight	Percentage (%)
Yogurt (kg)	54.24	49.90
Water (L)	36	33.12
Sugar (kg)	1.08	0.99
ST syrup (L)	17.36	15.97
Total	108.685	100

### Pilot Plant PEF System

The yogurt-based drink was processed by an integrated OSU-2C pilot plant PEF system (Qiu *et al.* 1998). The PEF system consisted of co-field continuous flow tubular treatment chambers, tubular heat exchangers, a 40kV/ 20kW high voltage pulse generator (Fig. 1), a cooling/temperature regulating system, fluid handling system, and an aseptic packaging machine (Yin *et al.* 1997). Each PEF treatment chamber, through which the food materials pass during the processing, consisted of two boron carbide electrodes and an insulator made of Delrin®

holding the electrodes in position as well as forming an enclosure for the food materials. The chamber diameter was 0.635 cm, and the gap distance between the electrodes was 1.0 cm. Four chambers were connected in series (electrically in parallel), thus enabling the products to flow sequentially through all the four chambers. In order to monitor the system temperatures, a series of sanitary Resistance Temperature Device (RTD) probes with dual sensing elements (Model R1T285L4801, Inotek, Bensenville, IL) were placed in short t-pieces at the inlet and outlet of PEF treatment chambers and at the outlet of the cooling system. The processing temperature was adjusted to 30°C by dual chillers (Model CFT-150, Neslab Instruments, Newington, NH).

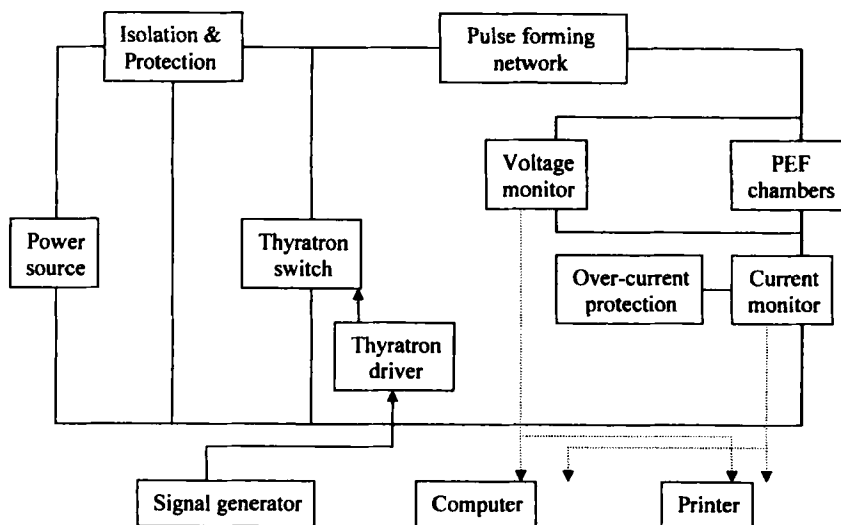


FIG. 1. SYSTEM DIAGRAM OF A PILOT PLANT-SCALE HIGH VOLTAGE PULSE GENERATOR

### Fluid Handling System

A sanitary fluid handling system provided the processing and transfer of the product to a packaging machine (Fig. 2). Prior to the processing, the entire system was sterilized in place (SIP) with a 30 min pressurized hot water cycle at 121°C. The product was then pumped from the stainless steel tank through the tubular heat exchangers to heat, hold and cool the product, respectively, using a Moyno progressive cavity pump (Model CFB 2C SSV3SAA, Moyno Industrial

Products, Springfield, OH). This pump provided a constant pulseless flow to facilitate a uniform thermal and PEF-treatment of the product.

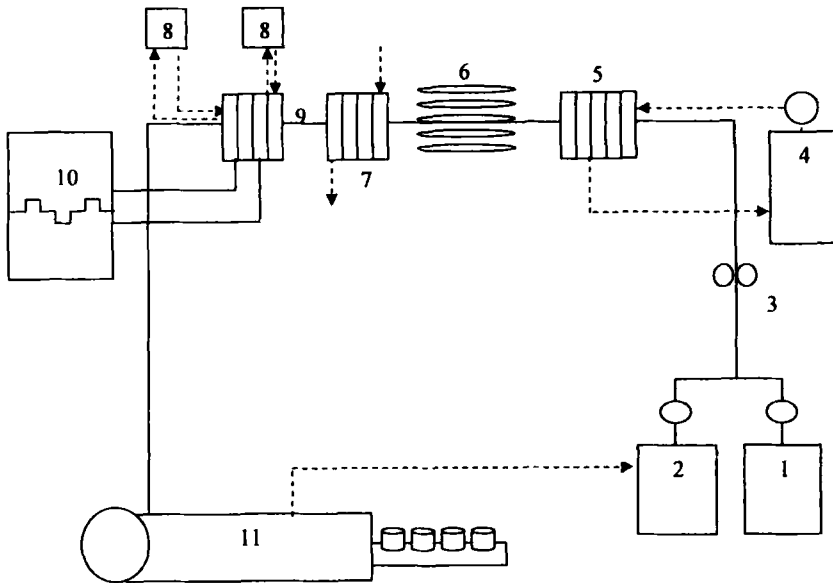


FIG. 2. CONFIGURATION OF PILOT PLANT PEF PROCESSING SYSTEM

Where 1. Product tank; 2. CIP/SIP tank; 3. Product pump; 4. Hot water heater; 5. Heating heat exchanger; 6. Holding tube; 7. Cooling heat exchanger; 8. Chillers; 9. Temperature-control heat exchanger; 10. Treatment chambers and PEF Pulse Generator; and 11. Aseptic packaging machine.

A magnetic flow meter (Model AM202AG, Johnson-Yokogawa, Newman, GA) was used to measure the treatment flow rate. A series of seven sanitary RTD probes were connected to a network module (National Instruments, Austin, TX) and the temperature data were captured by the network module and saved in a computer. Once processed, the product was pumped to an aseptic packaging machine that filled it into thermoformed cups.

### Processing of Yogurt-Based Drink

For the combined mild heat and PEF treatment, the prepared yogurt-based drink was heated at 60C for 30 s, cooled to 30C, and then PEF treated by using bipolar pseudo square waveform pulses, and aseptically filled. For the mild heat

treatment only, the yogurt-based drink was heated at 60C for 30 s, cooled to 30C and aseptically filled. PEF-treatment parameters were 30kV/cm electric field strength, 1.4  $\mu$ s pulse width, 500 pulse per second (pps) pulse repetition rate, and 32  $\mu$ s total treatment time. Flow rate was adjusted to 100 L/h. The control samples were the ones pumped through the integrated pilot plant system without any treatment and packaged by the packaging machine. The processing of the yogurt-based drink was duplicated under the same conditions.

### **Packaging Machine**

A Benco Aseptic packaging machine (Model Asepak/2, Placenza, Italy) was integrated with the pilot plant PEF system and equipped with steam, filtered water and hydrogen peroxide sterilization. The machine was used to package the treated and control yogurt-based drink samples. After the PEF-treatment, the yogurt-based drink samples were aseptically packaged in 180 mL tri-laminate plastic containers thermoformed by the packaging machine. The base material (Allista Plastic Packaging Co., Muncie, IN) consisted of high impact polystyrene (HIPS), polyvinylidene chloride (PVDC), and low-density polyethylene (LDPE). Container lid material was aluminum foil laminated with polyamide and LDPE.

### **Sampling**

The control and treated yogurt-based drink samples were packaged by the aseptic packaging machine in the 180 mL containers. A total of 300 containers were collected randomly from the start, middle and end of the treated and control samples coming out of the aseptic packaging machine. Likely variations in the treatment parameters during the process of having the treated and control samples pass through the packaging machine were, thus, reduced to a minimum. For 91 days, half of the containers were stored at 4C, and the other half at 22C. At certain time intervals (the days 0, 3, 7, 14, 21, 28, 42, 56, 70 and 91) of the 91-day storage, two containers from 4C and two from 22C for the treatments and the control were randomly picked for the shelf-life studies.

### **Microbiological Analysis**

Microbial growth in the treated and control samples during the shelf-life was measured through total plate count (TPC) and total mold and yeast counts. The samples were plated onto plate count agar (PCA) for TPC and onto potato dextrose agar (PDA) acidified with 10% tartaric acid for mold and yeast counts. The yogurt-based drink samples were diluted with 0.1% sterile peptone water, and the obtained dilutions were plated using a Spiral Plater (Autoplate model 3000, Spiral Biotech, Bethesda, MD). PCA plates were incubated at 37C for two days, and PDA plates at 25C for five days. PCA, PDA and peptone were

purchased from Difco Laboratories (Detroit, MI), and tartaric acid from Sigma (St Louis, MO).

### **Sensory Evaluation**

The control and treated yogurt-based drink samples were randomly collected and tested on the same day for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Silliker Laboratories Inc. (Columbus, OH), prior to the sensory evaluations. After the confirmation of the absence of the pathogenic microorganisms, the yogurt-based drink samples were kept in the refrigerator for 7 to 14 days and evaluated by an untrained sensory panel composed of 20 students from the Department of Food Science and Technology of the Ohio State University (Columbus, OH). The 60C+PEF treated and control yogurt-based drink samples stored at 4C were served in plastic cups to the panelists. Panelists evaluated each yogurt-based drink sample in terms of appearance, texture, flavor, taste, and overall acceptability, based on a nine-point hedonic scale of preference, ranging from 1 "dislike extremely" to 9 "like extremely."

### **Measurement of Color**

Color measurements of L, a and b values were performed in a 10 mm cell, using a Hunter Lab Ultra Scan colorimeter (Hunter Associates Laboratory, Reston, VA). The parameters "L", "a" and "b" are the measures of brightness/whiteness that varies from 0 to 100 (white if L = 100, black if L = 0), redness that varies from -a to +a (-a = green, +a = red), and yellowness that varies from -b to +b (-b = blue, +b = yellow), respectively.

### **Measurement of Physical Properties**

The physical properties of pH and °Brix (total soluble solids) were measured at room temperature. An ATI Orion pH meter (Model 370, Fisher Scientific, Pittsburgh, PA) was used to measure pH. A hand-held refractometer by Fisher Scientific (Pittsburgh, PA) was used to determine °Brix.

### **Statistical Analysis**

All statistical analyses were conducted with Minitab version 12.1 software (Minitab, Inc., State College, PA), using a significance level of 0.05. Two-way analysis of variance (ANOVA) for the sensory analysis, and paired t-test for the shelf-life analysis were performed to determine the statistical significance of the variation between the treatments. Response variables were microbial growth (PCA and PDA counts) as an indicator of the product safety, color retention, °Brix and pH as indicators of shelf-life quality of the product.

## RESULTS

### Effects of Combined 60C and PEF Treatment on Microorganisms

The shelf-life studies for 91 days at 4 and 22C demonstrated that the 60C+PEF samples had a significantly lower total plate count (TPC) than the control samples ( $P < 0.001$ ). After subjected to the 60C+PEF treatment, the yogurt-based drink samples had a  $4\text{-log}_{10}$  cfu/mL reduction in the initial TPC, relative to the control samples. After a 91-day storage at 4C, the 60C+PEF-treated samples had a TPC of  $3.27 \text{ log}_{10}$  cfu/mL, whereas the control samples reached a TPC of  $6.7 \text{ log}_{10}$  cfu/mL ( $P < 0.05$ ). TPC of the 60C+PEF samples stored at 22C was even lower than that of the control samples stored at 4C (Table 2 and Fig. 3).

TABLE 2.  
RESULTS FROM PAIRED T-TEST FOR TOTAL PLATE COUNT (TPC) AND TOTAL  
MOLD AND YEAST COUNTS OF THE YOGURT-BASED DRINK SAMPLES  
STORED AT 4 AND 22C

Microbial counts (log cfu/ml) after storage of 91 days	*60 C+PEF (Mean $\pm$ SD)	*Control (Mean $\pm$ SD)
TPC at 4 C	3.27 $\pm$ 0.07 <sup>a</sup>	6.7 $\pm$ 0.03 <sup>b</sup>
Total mold and yeast counts at 4 C	2.52 $\pm$ 0.11 <sup>a</sup>	4.03 $\pm$ 0.13 <sup>b</sup>
TPC at 22 C	4.78 $\pm$ 0.24 <sup>a</sup>	7.04 $\pm$ 0.19 <sup>b</sup>
Total mold and yeast counts at 22 C	3.31 $\pm$ 0.10 <sup>a</sup>	4.53 $\pm$ 0.08 <sup>a</sup>

\* Data in the same row with different superscript letters are significantly different ( $P \leq 0.05$ ).

Results of total mold and yeast counts of the control and 60C+PEF-treated yogurt-based drink samples stored at 4 and 22C for 91 days are shown in Table 2 and Fig. 4. At 4C, the variation in the total mold and yeast count between the 60C+PEF and control samples was significantly different ( $P < 0.05$ ). The control samples at 22C exploded by the day 42, and hence, the microbial counts for the samples could be performed by the day 42 only.



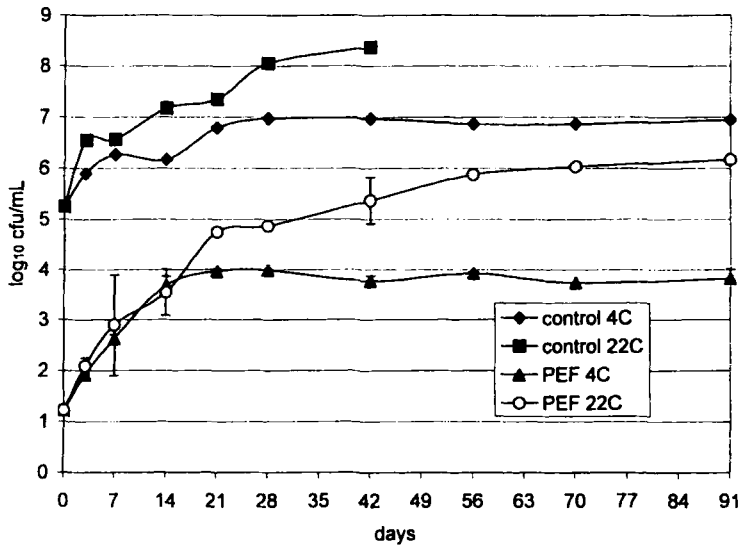


FIG. 3. RESULTS FROM TPC COUNT OF THE MILD HEAT (60C)+PEF AND CONTROL SAMPLES STORED AT 4 AND 22C

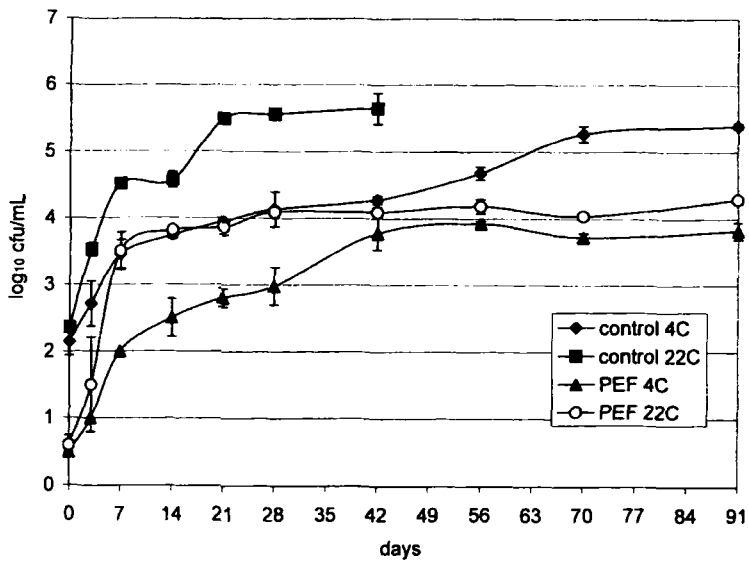


FIG. 4. RESULTS FROM TOTAL MOLD AND YEAST COUNT OF THE MILD HEAT (60C)+PEF AND CONTROL SAMPLES STORED AT 4 AND 22C

The mild heat-treated samples had no reduction in the initial TPC and total mold and yeast counts. These samples exploded in the first 14 days of the shelf-life study at 4 and 22C. Consequently, results from the samples for the TPC and total mold and yeast counts could not be compared with the control and 60C+PEF treated samples.

### Effects of Combined 60C and PEF Treatment on Color

Except for "a" value at 22C, the variation in the "L", "a", and "b" values for 4 and 22C between the control and 60C+PEF samples was not significant ( $P > 0.05$ ). The control samples had higher "L" and "b" values and a lower "a" value than the 60C+PEF samples, at 4 and 22C. However, this difference did not appear to be significant ( $P > 0.05$ ) (Table 3, Fig. 5). Color stability of the yogurt-based drink samples was affected more by the storage temperature than by the treatments. A higher storage temperature significantly lowered the "a" value of the yogurt-based drink samples ( $P < 0.05$ ). The control samples stored at 22C and 60C-treated samples at 4 and 22C exploded by the days 42 and 14, respectively. Color measurement for the control samples was performed for 42 days, whereas no color data could be obtained from the 60C-treated samples.

### Effects of Combined 60C and PEF Treatment on pH and °Brix

The initial pH and °Brix of the samples were not affected by the 60C+PEF treatment ( $P > 0.05$ ). The pH of the control samples was not significantly different from that of the 60C+PEF treated samples ( $P > 0.05$ ). Similarly, °Brix values of the control and 60C+PEF samples at 4 and 22C did not

TABLE 3.  
RESULTS FROM PAIRED T-TEST FOR PHYSICAL PROPERTIES OF THE  
YOGURT-BASED DRINK SAMPLES STORED AT 22C

Physical properties after storage	*Control (Mean± SD)	*60 C+PEF (Mean± SD)
of 91 days		
Color (L value)	64.79± 2.86 <sup>a</sup>	62.92±0.80 <sup>a</sup>
Color (a value)	21.19±1.23 <sup>a</sup>	23.71±1.65 <sup>b</sup>
Color (b value)	3.06±0.40 <sup>a</sup>	3.139±0.22 <sup>a</sup>
pH	3.79±0.30 <sup>a</sup>	3.89±0.22 <sup>a</sup>
°Brix	20.07±0.16 <sup>a</sup>	20.24±0.15 <sup>a</sup>

\*Data in the same row with different superscript letters are significantly different ( $P \leq 0.05$ ).

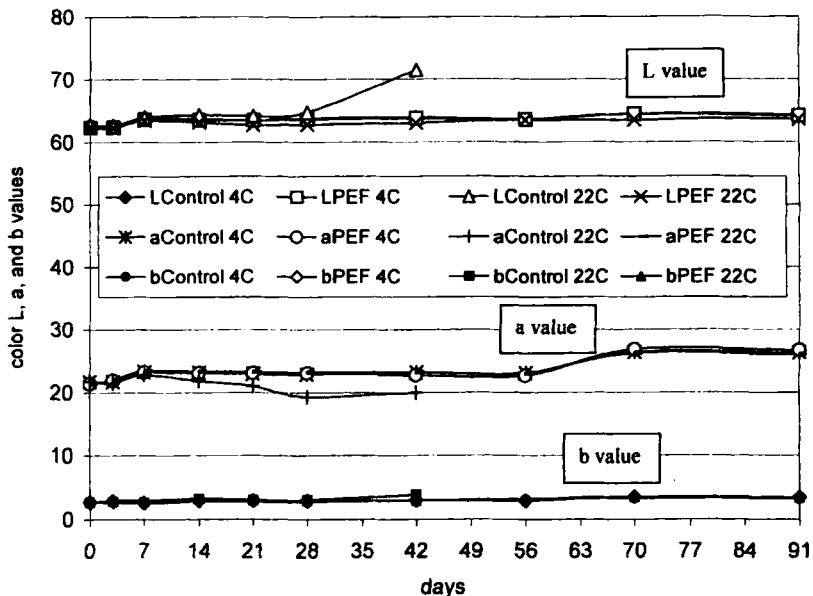


FIG. 5. RESULTS FROM COLOR MEASUREMENTS OF THE MILD HEAT (60C)+PEF AND CONTROL SAMPLES STORED AT 4 AND 22C

significantly differ ( $P > 0.05$ ). Decreases in pH value were observed for the control samples after 14-day storage at 22C and for the 60C+PEF samples after 70-day storage. The decreases in pH values at 22C for the control and 60C+PEF-treated samples were not significant ( $P > 0.05$ ) (Table 3, Fig. 6).

The decrease in the °Brix values for the 60C+PEF treated samples stored at 4C between the days 21 and 56 was not significant ( $P > 0.05$ ) (Fig. 7). The control samples stored at 22C exploded after 42-day storage. Therefore, the pH and °Brix measurements for these samples could be performed for 42 days only. Due to the explosion of the 60C-treated samples in 14 days of the shelf-life study, the measurement of their physical properties could not be performed.

#### Effects of Combined 60C and PEF Treatment on Sensory Properties

Sensory panelists indicated no significant difference in appearance, texture, flavor, and overall acceptability between the 60C+PEF treated and control samples ( $P > 0.05$ ). The 60C+PEF treated samples were ranked slightly higher than the control samples, but the difference was not significant (Table 4).

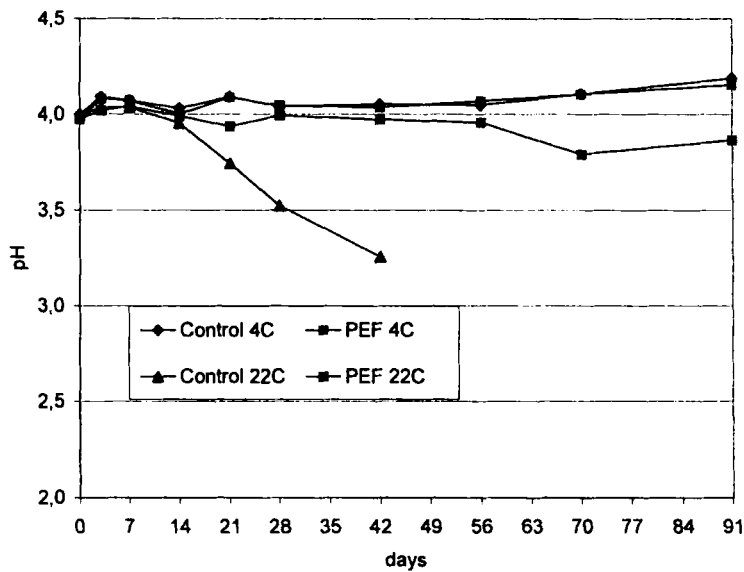


FIG. 6. RESULTS FROM pH MEASUREMENTS OF THE MILD HEAT (60C)+PEF AND CONTROL SAMPLES STORED AT 4 AND 22C

TABLE 4.  
RESULTS FROM ANOVA TEST FOR SENSORY ANALYSIS OF THE  
YOGURT-BASED DRINK SAMPLES STORED AT 4C

Selected sensory attributes	Control samples (Mean± SD)	60 C+PEF treated samples (Mean± SD)
Appearance	5.875±1.83 <sup>a</sup>	6.225±1.75 <sup>a</sup>
Color	5.825±2.18 <sup>a</sup>	6.025±2.09 <sup>a</sup>
Texture	5.775±1.85 <sup>a</sup>	6.175±1.75 <sup>a</sup>
Flavor	6.075±1.71 <sup>a</sup>	6.325±1.8 <sup>a</sup>
Overall acceptability	6.075±1.90 <sup>a</sup>	6.425±1.68 <sup>a</sup>

\*Data in the same row with same superscript letters are not significantly different ( $P\leq0.05$ ).

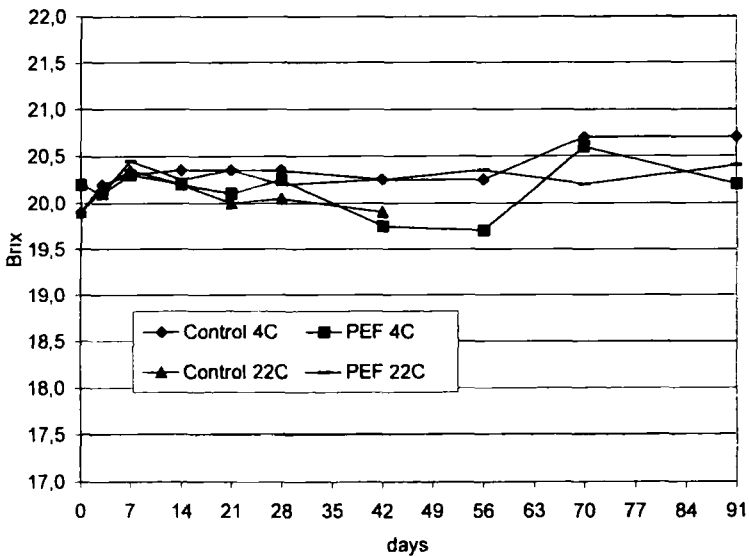


FIG. 7. RESULTS FROM °BRIX MEASUREMENTS OF THE MILD HEAT (60C)+PEF AND CONTROL SAMPLES STORED AT 4 AND 22C

## DISCUSSIONS AND CONCLUSIONS

Pumping and processing requirements limit the use of PEF technology for high viscosity food products such as yogurt-based drinks. PEF dosage and heating studies were conducted to find the PEF treatment parameters that can be applied to the yogurt-based drink without any arcing. Yogurt-based drink cannot be pumped through the fluid handling system without an initial heating, due to its viscosity. Therefore, the heating of the product was performed without adversely affecting the initial microbial flora. The heat treatment of 60C for 30 s with 100 L/h flow rate was observed to be enough to pump the product without a significant reduction in the initial microbial flora.

Previous studies indicated that the number of pulses per chamber of at least 2, the pulse duration of at least 2  $\mu$ s and the electric field strength of at least 15-20 kV/cm must be applied in order to inactivate microorganisms (Zhang *et al.* 1994a, b). Out of the above factors, the electric field strength was a relatively more determining factor in the microbial inactivation (Knorr *et al.* 1994; Qin *et al.* 1995). From the PEF dosage study, the optimum PEF processing parameters were found to be 30 kV/cm electric field strength, 1.4  $\mu$ s pulse width, 500 pps

pulse repetition rate, 100 L/h flow rate, and 32  $\mu$ s total treatment time. These initial studies revealed that the application of 60C for 30 s with the combination of the selected PEF parameters could extend the shelf-life of the yogurt based drink. It also should be noted that when combined with the PEF treatment, the heat treatment at 60C for 30 s might have a synergistic effect on the microbial inactivation (Jayaram *et al.* 1992). On the other hand, the shelf-life study of the heat-treated samples showed that all 60C for 30 s samples spoiled in 14 days.

Yogurt is not a shelf-stable product in that the shelf-life of yogurt is limited up to 3 weeks even under refrigeration (Tamime and Deeth 1980). However, this study demonstrated that a pilot plant scale PEF-processing system in combination with mild heat treatment and aseptic packaging might be used to effectively extend the shelf-life of the yogurt-based drink. At the end of the 91-day storage at 4C, both TPC count and total mold and yeast counts of the samples treated by 60C+PEF were less than 4 log cfu/mL (Table 2). Not only did the 60C+PEF treatment achieve a longer shelf-life but also the physical and sensory properties of the 60C+PEF samples did not deteriorate relative to those of the control samples (Fig. 5, 6 and 7). These findings support the scientific literature that the mild heat combined with PEF can achieve the necessary microbial inactivation with less detrimental organoleptic consequences for the high viscosity food products.

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